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Reviews

Double Umbilical Cord Blood Transplantation: Relevance of Persistent Mixed-Unit Chimerism

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Double umbilical cord blood transplantation (UCBT) was developed as a strategy to circumvent the cell dose limitation of single UCBT with a concomitant potential benefit of lowering the rate of leukemia relapse. Sustained hematopoiesis after double UCBT usually is derived from a single donor unit, as only a few patients have been reported to display stable mixed-unit chimerism for varying periods of time. Explanations for the 1 unit dominance, predictors for identifying unit superiority, and persistence of long-term mixed-unit chimerism remain elusive. Review of published literature revealed only 11 of 280 patients (4%) with mixed-unit chimerism for at least 1 year after transplantation, with 3 patients receiving reduced-intensity conditioning regimens. Mixed-unit chimerism was more likely if both units were closely HLA matched to each other. Outcome data for patients with stable mixed-unit chimerism, for the most part, were scarcely reported. Analysis of the small sample size revealed a potential advantage of stable mixed-unit chimerism on enhancing the graft-versus-leukemia effect; however, definitive conclusions cannot be made on the effect of mixed-unit chimerism on the rates of graft-versus-host disease. Therefore, gathering outcome data prospectively in larger clinical series will help answer the question of whether stable mixed-unit chimerism is either beneficial and, therefore, should be strived for, detrimental and, thus, needs to be eliminated, or if it is of no clinical consequence.

INTRODUCTION

Hematopoietic progenitor cells isolated from umbilical cord blood (UCB) are an alternative graft source for allogeneic hematopoietic cell transplantation (AlloHCT) for those patients lacking a suitable histo-identical sibling or a well-matched adult unrelated donor. UCB has the advantage of being readily available with a less stringent requirement for human HLA matching because of the immunologic naiveté of UCB cells and the reduced numbers of lymphocytes in the unit [1,2]. This graft source has become a standard therapeutic option for pediatric hematologic malignancy patients and the result of using UCB compares favorably with unrelated blood and bone marrow grafts for AlloHCT [3]. In larger children and adults, however, UCB transplantation (UCBT) efficacy is severely limited by the low progenitor cell dose per recipient weight, leading to high risk of delayed or failure of engraftment [4].

Various strategies to overcome this drawback include the use of double (ie, dUCBT) rather than single-unit UCBT, ex vivo expansion of UCB units, direct intrabone marrow injection, and use of agents to enhance cell homing [5-8]. These interventions have been met with limited success. Despite over a decade of using these approaches, no single technology has emerged as a preferred approach. In the vast majority of dUCBT cases, sustained engraftment of only 1 donor unit ultimately dominates and the other unit no longer can be detected [9,10]. In rare cases, long-term hematopoiesis can be observed from both donor units in varying ratios, a condition referred to as *mixed-unit chimerism*. To date, no factors have been identified in these cases that reliably predict which unit will emerge as the dominant unit. The mechanism for such single-unit dominance remains to be elucidated [9,10]. The study of dUCBT is of even greater interest given recent reports suggesting that dUCBT may be associated with a reduced risk of leukemia relapse, which is thought to possibly be a result of unit-to-unit allogeneic interactions [11,12].

Chimerism results after transplantation are significant. In patients with malignant diseases, chimerism is primarily used to detect early disease relapse, but it can also indicate

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Table 1
Clinical Studies Addressing Single-Unit Chimerism

Author, yr	Patient, n	Age, Median (Range), yr	Disease	Regimen	TNC Dose, $\times 10^7$/kg	CD34 dose, $\times 10^5$/kg	Chimerism Pattern	Comment
Barker, 2005 [14]	23	24 (13-53)	ALL, AML, CML	MAC	Dominant: 1.8 Non-dom: 1.9	Dominant: 1.5 Non-dom: 2.5	1 unit dominates in all patients by d 100	Higher $CD3^+$ dose was significantly associated with SUD (P value <.01); however, differences in $CD3^+$ cell dose were minimal (.6 $\times$ 10^7 versus .4 $\times$ 10^7)
Kang, 2006 [17]	8	14 (6-17)	AML, ALL, SAA	MAC	4.68	1.75	SUD by d +28 in all patients	Selection of 1 strong unit (with high numbers of total nucleated and/or $CD3^+$ cells) and 1 weak unit to activate strong 1; thus creates SUD, might result in better outcome
Ballen, 2007 [15]	21	49 (24-63)	AML, NHL, CLL, MDS, ALL	RIC	Dominant: 2.2 Non-dom: 1.8	Dominant: 1.8 Non-dom: .7	Dominance of 1 UCB unit at 3 mo in all patients	First unit infused more often dominant; 90% of the units infused 4 hr apart
Brunstein, 2007 [16]	93	51 (17-69)	ALL, AML, MDS, NHL, CML, HL	RIC: 1/3 given ATG	3.7	4.9	Early transient MUC followed by SUD at d 100	No factors reliably predict which of 2 UCB units predominate
Yin, 2011 [21]	12	31 (17-42)	AML, ALL, CML	MAC, all given ATG	Dominant: 2.85 Non-dom: 2.5	Dominant: .55 Non-dom: .25	SUD in all patients at 1 yr	Neither TNC nor $CD34^+$ cell doses correlated with dominance
Wallet, 2013 [22]	136	38.5 (9-63)	ALL, AML, CML, CLL, NHL, MM	MAC: one third RIC: two thirds, one quarter given ATG	3.1	1.4	Unit gender-matched with recipient more likely to become dominant	Disease status at time of transplant remains the major prognostic factor for outcome
Song, 2013 [23]	29	23 (10-48)	AML, CML, ALL, MDS	MAC: one fifth given ATG	4.7	2.4	1 transient MUC for short time	Neither TNC nor $CD34^+$, $CD3^+$ or GM-CFU affected unit dominance
Kai, 2013 [24]	61	37 (10-54)	ALL, AML, CML, MDS, NHL	MAC	3.53	1.04	All patients had SUD by d +60	Only degree of HLA disparity in host-versus-graft direction affected SUD

TNC indicates total nucleated cell; ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; Non-dom, nonpredominant unit; SUD, single-unit dominance; SAA, severe aplastic anemia; NHL, non-Hodgkin lymphoma; CLL, chronic lymphocytic leukemia; MDS, myelodysplastic syndrome; HL, Hodgkin lymphoma; MUC, mixed-unit chimerism, MM, multiple myeloma.

impending rejection. In patients with nonmalignant disorders, it is merely used to monitor successful engraftment. The ability to detect the dominant unit early after transplantation might be useful clinically, as delayed hematopoietic recovery and immune reconstitution after dUCBT remain ongoing limitations to more widespread adoption of the approach using UCB as a donor graft source. Moreover, lack of available donor lymphocyte infusion in UCBT (as potential adoptive immunotherapy to improve engraftment or to treat relapse) is problematic. Here, we review the dUCBT literature regarding the frequency of mixed-unit chimerism occurrence, the clinical outcomes, and implications for UCB graft selection.

METHODS

We undertook a PubMed literature search for relevant clinical trials and reviews (from January 1, 1985 to April 1, 2014) using the following key words: umbilical cord blood, transplantation, double, mixed chimerism, and dominance. We used those key words in different combinations. We focused on the studies that are related to our review subject of dominance and mixed-unit chimerism in the setting of dUCBT. We also cross-referenced review articles but focused on clinical studies and some preclinical trials, regardless of patient ages and minimum numbers of patients in a trial or report.

After successful AlloHCT, the recipient usually adopts the donor hematopoietic system and becomes a full donor chimera. In some cases, however, recipient hematopoietic cells remain and the patient instead becomes a mixed chimera. *Split chimerism* is used when the coexistence of donor and recipient cells is observed in specific cellular lineage but not in others. In the current article, we focused on hematopoietic progenitor cell chimerism. A patient is considered to be *a mixed chimera* if he or she has 5% to 95% of hematopoietic cells of donor origin [13]. After a period of transient engraftment of both UCB units, a single unit emerges as the "winner" to sustain long-term hematopoiesis, ie, at least 90% marrow reconstitution by donor cells [14-16]. The time frame for determining dominance has not yet been clarified [17]. Usually, by day 21 after transplantation, single-unit dominance can be detected in over 80% of patients, although dominance as soon as 14 days after transplantation has been reported [10,18]. Sustained detection of both UCB units in varying ratios over 21 days generally is termed *mixed-unit chimerism*. Dominance reversion occurs when the fraction of cells of the predominating UCB unit decline gradually and give up dominance to the other unit in the state of mixed-unit chimerism. In an analysis of 23 dUCBT after myeloablative conditioning (MAC), hematopoiesis was observed from a single donor in 76% patients at day 21 and in 100% by day 100 after transplantation [14]. Likewise, a review of 81 dUCBT after a nonmyeloablative (NMA) regimen, single-donor chimerism was detectable in 57%, 81%, and 100% of patients at day 21, 100, and 365, respectively [16].

Chimerism is often determined using bone marrow or blood samples obtained at 21, 60, 100, 180, 360, and 720 days after transplantation, with the use of additional time points as clinically indicated. Methods and approaches for chimerism monitoring after dUCBT are discussed in detail by Kristt et al. [19].

SINGLE-UNIT DOMINANCE

Although single-unit dominance has been well described, prior studies have not identified the mechanism or a reliable method of predicting which will be the long-term engrafting unit [9,10]. Verneris et al. stated "predicting the winning unit seemed impossible and more like atmospheric noise" [20]. However, other investigators continue to attempt to define predictors of UCB unit dominance. Gutman et al. showed evidence that donor T cells from the engrafting UCB unit specifically recognize the nonengrafting unit [18]. Taking into consideration the intrinsic properties of the 2 infused units and the immune interactions between the recipient and the donor units, some studies have attempted to assess whether single-unit dominance is influenced by the intrinsic features of the UCB units. Table 1 depicts 8 clinical reports addressing single-unit chimerism. The studies contained 8 to 136 patients; the majority of subjects received MAC regimens and the most frequent disease indication was acute

Table 2
Candidate Factors Involved in Single-Unit Chimerism

Candidate Factors	Supporting Citations	Conditioning Regimen	*P* Value
$CD3^+$ cell dose	Barker [14]	MAC	<.01
	Avery [25]	MAC	.04
	Ramirez [26]	MAC + NMA	<.01 + .05
	Scaradavou [27]	MAC + NMA	.0077
	Kanda [28]	MAC	.042
$CD34^+$ cell dose	Avery [25]	MAC + NMA	.008
	Scaradavou [27]	MAC + NMA	.0016
	Haspel [29]	RIC	<.01
GM-CFU	Brunstein [16]	RIC	.02
	Yoo [30]	MAC	.006
HLA match (donor-recipient)	Kai [24]	MAC	.0218
	Ramirez [26]	NMA	.05
Order of i.v. infusion	Ballen [15]	RIC	.049
	Haspel [29]	RIC	.03
Route of infusion (i.v. versus intramarrow)	Brunstein 6 (No advantage of intramarrow over i.v. infusion for unit dominance)	MAC	NS
Gender match	Wallet [22]	MAC + RIC	.094
HLA match (donor-donor)	Avery [25] (closely HLA-matched units to each other were most likely to have initial and sustained transient chimerism)	MAC + NMA	<.0001
TNC dose	Kanda [28]	MAC	.02
	Haspel [29]	RIC	.01

NS indicates not stated.

leukemia. Not 1 identified factor can reliably predict the dominant unit. In the majority of patients, transient dual chimerism was replaced by sustained dominance of 1 unit by 100 days after dUCBT, regardless of whether a patient received MAC or reduced-intensity conditioning (RIC).

Factors Predictive for Single-Unit Dominance

A variety of specific factors have been examined for the ability to reliably predict which unit will emerge as dominant [9,10]. Table 2 lists some of the candidate factors, including cell viability, infused total nucleated cell count, $CD34^+$ or $CD3^+$ cell doses administered, gender matching between recipient and graft, ABO blood group incompatibility, degree of HLA mismatch between the recipient and each unit or between the 2 units, order of UCB unit administration, and route of infusion (i.v. versus intraosseous) [6,9,14,15,22,24-30]. Despite most of the candidate factors being statistically significant in determining unit dominance (as indicated by *P* values listed in the same table), no single factor can be identified as being consistently responsible (shown below).

Cellular characteristics of the graft

Barker and colleagues [14] previously reported that the UCB unit with the higher $CD3^+$ cell dose was more likely to predominate. The differences in $CD3^+$ cell doses, however, were minimal, as the median infused $CD3^+$ dose of the predominating unit was $.6 \times 10^7$ $CD3^+$/kg compared with $.4 \times 10^7$ $CD3^+$/kg of the nonsustained unit ($P < .01$). Ramirez et al. [26] also showed the $CD3^+$ dose to be associated with single-unit dominance regardless of the conditioning regimen employed. Avery et al. [25] described an association between $CD3^+$ cell dose and $CD34^+$ cell viability, in which the dominant unit was more likely to be characterized by a higher infused $CD3^+$ cell dose and $CD34^+$ cell viability > 75%. Brunstein et al. [16] noted no difference between dominant and nonsustained units in terms of total nucleated cell, $CD34^+$, and $CD3^+$ cell doses, but they described a higher granulocyte-macrophage colony-forming unit (GM-CFU) in the dominant unit. Yoo et al. [30] also showed GM-CFU to be the only significant factor predicting engraftment of the dominant unit. Finally, Scaradavou et al. [27] suggested that units with low $CD34^+$ cell viability (<75%) are less likely to engraft upon coinfusion with a unit with high cell viability (>75%). As noted above, some but not all studies were able to show that $CD3^+$ cell doses, $CD34^+$ cell doses, and GM-CFU were able to predict the dominant unit. These data demonstrate that cellular characteristics and cell dose/unit selection, to date, are not reliable in predicting single-unit dominance, ie, the intrinsic features of the graft are difficult to manipulate.

Degree of HLA match

The Minnesota group [14,25,27] reported that disparity between the UCB unit and the recipient does not affect dominance, whereas a closer HLA match between both units is associated with an initial engraftment and transient persistence of both units [25]. Ramirez et al. [26] showed, only in the NMA setting, that better unit-recipient HLA match was an independent factor associated with single-unit dominance. It is possible that the influence of HLA matching in the setting of NMA conditioning could be due to residual host T cells interacting with UCB T cells; in the MAC setting, these host cells will have been eradicated or rendered nonfunctional. More recently, Kai et al. [24] analyzed 61 hematologic malignancy patients who underwent dUCBT after MAC. A lower degree of HLA disparity in the host-versus-graft direction was associated with unit dominance. Again, HLA match between UCB units and recipient was not a reliable predictive factor of single-unit dominance regardless of the conditioning regimen. Alternatively, Avery et al. [25] reported an interesting observation that closer HLA match between both UCB units is associated with initial engraftment of both units and the transient persistence of the nondominant unit (*P* value <.0001; see below).

Order, route of infusion of UCB units, and gender matching

Two groups reported that when infusing 2 different UCB units intravenously 3.5 to 4.5 hours apart, the dominant unit usually was the unit infused first [15,29]; the order of infusion loses its significance when units are infused sequentially without a hiatus. Brunstein et al. [6] showed that neither

intravenous nor intraosseous administration of UCB units were shown to confer a selective advantage in unit dominance. Data from the SFGM-TC registry did not demonstrate statistical significance of gender matching with the recipient in determining the dominant unit. This group did note, however, that patient survival was improved after male UCB engraftment among male recipients [22].

Conclusions for factors predictive of single-unit dominance

Although the above data indicate that several candidate factors intrinsic to UCB units may suggest development of unit dominance, no single factor was identified as being consistently responsible.

Mechanisms for Single-Unit Dominance

The observation of increased acute graft-versus-host disease (GVHD) and decreased relapse rates after dUCBT suggest that immunologic interactions may underlie the emergence of a winning unit. Additional immune interactions between the recipient and both donor units, as well as graft-versus-graft immune interactions (to be discussed below) may play a greater role in this process.

Although it is unclear if the data generated using a xenograft immunodeficient murine model will be predictive, several preclinical studies suggested that an immune-mediated effect could play a role in promoting the dominance of 1 UCB unit [31-34]. Clinical studies strongly support the importance of T cells to establish chimerism and ensure hematopoietic cell engraftment [35]. As discussed above, the concept that T cells also mediate dominance after dUCBT has been described, but a consensus has yet to be reached [14,18,24,36]. Few studies suggested graft-versus-graft immune interactions as a mechanism for single-unit dominance. Studies using immunodeficient murine xenograft models reported that donor T cells exert effects via interactions with donor progenitor cells and their microenvironment after they showed that the addition of T cells enhanced engraftment when compared with T cell–depleted mononuclear cells [33]. Kim et al. showed that single-unit dominance was observed after cotransplantation of mononuclear cells. On the other hand, cotransplantation of culture-expanded third-party mesenchymal stem cells results in more balanced coengraftment after dUCBT, which can be partly, if not completely, attributed to a reduced extent of donor deviation between the 2 grafts [31]. Yahata et al. found that $CD34^-$ cells mediate unit dominance, specifically by a combination of $CD4^+$ and $CD8^+$ cells, indicating that unit dominance is an immunologic phenomenon [37]. Other supporting data for a graft-versus-graft immune interaction for unit dominance come from in vitro data by Moretta et al. [36], who hypothesized that the dominant unit is able to develop cytotoxic activity directed against activated lymphocytes present in the other unit. The nondominant unit is, thus, prevented from exerting alloantigen-specific cytotoxic potential against both activated lymphocytes and hematopoietic progenitor cell content of the dominant unit. Other groups have suggested that the potential natural killer (NK) alloreactivity of 1 unit might contribute to unit dominance [38,39]. In conclusion, these results support the importance of graft-versus-graft cell-mediated alloreactivity as a principal mechanism promoting engraftment and single-unit dominance. Trying to validate this strategy prospectively in a clinical setting by applying 2-way mixed lymphocyte culture may be helpful in predicting the dominant unit.

Gutman et al. [18] provided compelling evidence that effector $CD8^+$ T cells may play a critical role in the predominant unit actively rejecting the losing unit. They postulated that decreased relapse might be related to the early post-transplantation immunologic interactions between the 2 infused units and residual host cells. If the dominant unit could be predicted in advance, a well-matched, smaller unit might be paired for infusion with a larger unit, both to facilitate engraftment of the better matched unit and enhance the graft-versus-leukemia (GVL) effect.

Newell et al. [40] showed a correlation of high early post-transplantation donor $CD3^+$ cells with the dominant unit, suggesting rapid immune-mediated response. The same group also demonstrated that infusion of a greater total $CD3^+$ cell dose, particularly of naïve $CD3^+/CD8^+$ T cells, may play an important role in determining single-unit dominance after dUCBT [41].

In conclusion, all previous clinical studies propose graft-versus-graft immune interactions as an immune-mediated mechanism of unit dominance. The mechanism of single-unit dominance is likely multifactorial, involving intrinsic features of the UCB units (summarized in Table 2) as well as graft-versus-graft interactions and possibly host-graft interactions. Identifying the mechanism for how 1 unit predominates could facilitate manipulation of UCB units by upregulating immune reactivity of 1 unit. Moreover, understanding graft-versus-graft interactions may shed the light on the host-graft interactions and, consequently, better understanding of GVL and GVHD.

MIXED-UNIT CHIMERISM

Table 3 depicts some of the reports illustrating the persistence, for varying time periods, of both UCB units (ie, mixed unit chimerism). The vast majority of prior reports show emergence of single-unit dominance early (by 60 days) and clearly by 12 months after transplantation. The data in Table 3 indicate the existence of a stable, mixed-unit chimerism state in some patients at 1 year after dUCBT. We suggest the use of a more strict definition for stable mixed-unit chimerism, ie, that condition in a patient in whom both donor units are detectable 12 months after dUCBT. Before 12 months after transplantation, we suggest this clinical situation be termed *transient mixed-unit chimerism,* as there remains a chance of skewing toward single-unit dominance. This practice should help provide standardize reporting of such patients in the future.

Dominance reversion is another novel situation that occurs when the fraction of cells of the predominating UCB unit decline gradually and give up dominance to the other unit in the state of mixed-unit chimerism. One case reported is a 15-year old boy who received a dUCBT for acute lymphoblastic leukemia. He developed long-term mixed-unit chimerism; later, dominance reversion from unit B to unit A occurred as assessed on day 253 after transplantation and was observed to persist during follow-up of more than 16 months (497 days) later [42]. In a phase I dUCBT clinical trial involving patients with hematologic malignancies RIC regimen, another patient with 95% single donor chimerism on day 65 after transplantation was reported to lose single-unit chimerism in favor of mixed-unit chimerism over time. By day 177 after transplantation, the last chimerism result showed the dominant UCB unit contribution to hematopoiesis dropped to 86% with the remaining 14% of hematopoiesis came from the second UCB unit [29]. The patient died

Table 3
Clinical Studies Reporting Assessment of Mixed-Unit Chimerism

Author, yr	Patients, n	Age, Median (Range), yr	Disease	Regimen; No of Patients	TNC Dose, $\times 10^7$/kg, and CD34 Dose, $\times 10^5$/kg	Neutrophil and Platelet Engraftment, Median, d	Outcome	Chimerism Pattern	Comment
Haspel, 2008 [29]	38	50 (18-65)	AML, NHL, ALL, CLL, HL	RIC (all received ATG)	Dominant: 2.18 Non-dom: 1.88 Dominant: 1.07 Non-dom: .56	20 43	No outcome data	2 MUC at 16 and 24 mo	Higher TNC and $CD34^+$ cell dose are associated with unit dominance
Yen, 2008 [42]	1	15	ALL	MAC (all received ATG)	A: .87 B: 1.79 A: .31 B: .49	35 59	Disease free; No GVHD	1 MUC at 16 mo (DR from unit B to unit A occurred at 170 d	First patient with DR in the setting of MUC.
Berglund, 2009 Gertow, 2010 [43,44]	7	30 (20-59)	AML, ALL, NHL	MAC (5) RIC (2) (all received ATG)	3.4 1.1	31 53	Both patients are doing clinically well with no GVHD	2 MUC at 28 and 45 mo	Unit-unit HLA-C match combined with ATG increase likelihood of MUC
Arachchillage, 2010 [45]	1	21	AML-M3 with CNS disease	MAC	A: 4.89 B: 3.2 NS	44 67	No GVHD; No severe infection	1 MUC at 28 mo	MUC may contribute to successful outcome
Kang, 2010 [46]	61	9 (1-18)	ALL, AML, SAA, JMML	MAC (46 received ATG)	Dominant: 2.45 Non-dom: 2.55 Dominant: 1.16 Non-dom: .91	18 46	4-yr EFS 57%; TRM 25% (mainly CMV); aGVHD II-IV 52%	1 MUC at 1 yr	Lower cell dose difference (<15%) may decrease EFS and increase TRM
Kanda, 2011 [28]	27	33 (20-85)	ALL, AML, CML, MDS	MAC	4.3 1.2	24 37	2-yr DFS 52%; 2-yr TRM 28%; aGVHD 37%	2 MUC at 2 yr	Higher TNC and $CD3^+$ were associated with the dominant unit
Avery, 2011 [25]	84	36.5 (.9-66)	AML, ALL, CML, CLL	MAC (61) NMA (23)	Dominant: 2.1 Non-dom: 2.3 Dominant: .9 Non-dom: .8	23 NS	No outcome data	1 MUC at 1 yr	Closer unit-unit HLA match is associated with MUC
Yadav, 2013 [47]	1	2	JMML	MAC	A: 7.1 B: 6.4 NS	15 49	Grade III delayed aGVHD; CMV infection	1 MUC at 9 mo	Persistent of both units is more likely if 6/6 match to each other
Milano, 2013 [41]	60	38 (18-52)	ALL, AML	MAC (46) RIC (14)	Dominant: 2 Non-dom: 1.9 Dominant: .12 Non-dom: .11	MAC 25 NS RIC 13 NS	No outcome data	1 MUC at 1 yr; MAC; 6/6 to each other	Higher $CD3^+$ dose is associated with unit dominance

DR indicates dominance reversion; A, unit A; B, unit B; CNS, central nervous system; EFS, event-free survival, JMML, juvenile myelomonocytic leukemia; TRM, treatment-related mortality; CMV, cytomegalovirus; aGVHD, actute graft-versus-host disease; DFS, disease-free survival.

1 month later from post-transplantation lymphoproliferative disorder.

Table 3 shows 11 of 280 patients (4% of the total in the studies) with mixed-unit chimerism at least 1 year after transplantation; another patient was reported at 9 months after transplantation. Three patients received RIC, although as noted above, NMA conditioning is more often associated with a period of mixed chimerism than MAC [16]. Although there was a small sample size, it appears that mixed-unit chimerism may be more prevalent with MAC. Gathering such information prospectively from larger series will facilitate addressing this question. Additionally, for the 2 patients who attained mixed-unit chimerism at 9 months and at 12 months after transplantation, both donor units were 6/6 HLA matched to each other; in a third patient, the UCB units were at least 5/6 HLA matched to each other [25,41,47]. For those 3 patients, 1 developed delayed, severe acute GVHD, whereas no data were reported for the other 2 patients, making it hard to reach any conclusions on the advantage of unit-to-unit HLA match. Such data are in agreement with Ponce et al. [48], who showed no advantage of unit-to-unit HLA matching from the standpoint of acute GVHD. Although continued study is appropriate, no data support the consideration of unit-to-unit HLA match in UCB unit selection at this time. Outcome data on those patients with mixed-unit chimerism are not reported for the most part. Exceptions include 3 case reports; 2 patients did not have GVHD [42,45] and a third developed delayed acute grade III skin and gut GVHD [47]. All 3 patients had resistant leukemias (high-risk acute promyelocytic leukemia, juvenile myelomonocytic leukemia, and very high-risk acute lymphoblastic leukemia) but were in remission at the time of report at 28, 9, and 16 months after dUCBT, respectively. Despite the very small sample size, their reports showed that an enhanced GVL effect is possible; however, one cannot draw conclusions from these studies on the rates of GVHD.

Berglund et al. [44] and Gertow et al. [43], respectively, used flow cytometry and cytokine production to analyze the phenotype and functionality of different cell subsets in cord blood units in 2 patients with mixed-unit chimerism; they showed that the 2 stable chimerism cord blood units are different phenotypically and functionally. In comparison with control patients having a single dominant UCB unit, those 2 mixed-unit chimerism patients had more naïve T cells and fewer $CD45RO^{+}CCR7^{-}$ and $CD4^{+}$ and $CD8^{+}$ memory T cells. These observation are in agreement with Gutman et al. [18] and Yahata et al. [37] who showed that unit dominance is mediated by a process that requires both $CD4^{+}$ and $CD8^{+}$ cells, through a process that likely requires either direct recognition of the MHC antigens or other nonspecific effects.

Antithymocyte Globulin and HLA-C Matched UCB Units

Berglund et al. [44] observed long-term mixed unit chimerism for more than 2 years in 2 of 7 patients, all of whom received high-dose antithymocyte globulin (ATG, 8 mg/kg) in the course of myeloablative alternative donor transplantations. They proposed that the high-dose ATG facilitated tolerance between both cord units via T cell depletion induced by ATG within already immunologically naïve cord blood cells. The same group extended their hypothesis by suggesting that HLA-C match and possible NK cell tolerance between both units in combination with high-dose ATG increases the likelihood of stable mixed-unit chimerism [43,49]. As T cells after dUCBT both reconstitute more slowly compared with adult hematopoietic cell sources and usually are present at a much lower overall number, the addition of a high-dose ATG greatly will reduce the potential for T cell–mediated rejection in any direction for a prolonged period of time [50]. The lack of T cells allows for NK cells to expand more freely. This could lead to tolerance in an HLA-C matched situation between both units, as NK cell function is regulated partly by inhibitory killer cell immunoglobulin-like receptors (KIRs) that recognize certain HLA-C molecules [36,51]. KIR-ligand mismatching between donor and recipient has been shown to be beneficial for a GVL effect in haploidentical cell transplantation [38]. Further, with dUCBT, KIR-ligand mismatch has been associated with a lower relapse rate as well as better overall survival [39]. Moreover, reduced relapse risk also has been reported for dUCBT in comparison to single UCBT [11]. The roles of KIR and KIR-ligand in the context of hematopoietic cell transplantation have been investigated over the past 10 years. In transplantations that are KIR-ligand mismatched in the graft-versus-host direction, donor NK cells expressing inhibitory KIRs, which do not recognize ligands on recipient targets, are released from HLA inhibition and lead to less restrained, more active NK cells. This situation results in clinically significant GVL effects. Better donor selection through high-resolution HLA typing, including HLA-C, not routine at the current time, may improve outcomes through enhanced GVL effect [52].

In conclusion, studies showed that HLA-C match between both donor units would increase the likelihood of stable mixed-unit chimerism through tolerance induction between both UCB units. Further, HLA-C mismatch between recipient and donor will increase the chance of alloreactivity, as they will not tolerate each other. Exploiting this NK cell effect to enhance GVL could improve patient outcome after dUCBT.

SUMMARY AND FUTURE DIRECTIONS

To date, no factors reliably predict for single-unit dominance or the coexistence of both units. The mechanism of single UCB unit dominance has been investigated by many groups and is most likely multifactorial. Specifically, this condition appears to involve intrinsic features of the UCB units, such as homing properties and proliferative potential of hematopoietic cells, graft-versus-graft immune interactions mediated by T cells, as well as graft-versus-host immune interactions [53-55]. Understanding those immune interactions may help us predict the dominant unit, and more importantly, could yield insights into the mechanisms of GVHD and GVL responses and improve outcomes. Regardless of these mechanistic questions, there remains the issue of how this information might be used to benefit patients and improve clinical outcomes. For instance, is it possible to reliably predict the “winning” unit in the future, and if so, why might we want to do so? If the dominant unit could be predicted, a well-matched smaller unit might be paired with a more poorly matched larger unit to facilitate engraftment of the better matched unit; perhaps the battle for dominance might enhance GVL while the ultimate dominance of a well-matched unit might lead to decreased GVHD and treatment-related mortality. One area of interest, but with a paucity of data in the literature, is the effect of the type, intensity, and timing of the immune suppression regimen or the timing of immune suppression withdrawal on mixed-unit chimerism. This maneuver may or may not contribute to the rapidity and severity of mixed-unit chimerism or single-unit dominance. The Minnesota group

has preliminary data on this subject. They believe that prolonged mixed double UCB chimerism is a reflection of 2-unit tolerance; the relapse rate appears increased in mixed chimeras because of reduced alloreactivity [56]. Regarding immune suppression withdrawal, this approach often differs significantly among centers and we are not aware of studies examining immune suppression withdrawal and chimerism. Given the wide variations in approach, it is quite difficult to reach conclusions at this time. This concept is an important question to explore in future studies and collaboration of centers that perform dUCBT would help answer that question and also would allow a standardized analysis of chimerism, given current differences between centers. The delayed hematopoietic recovery and immune reconstitution after dUCBT remain ongoing limitations and lack of available donor lymphocyte infusions, as potential adoptive immunotherapy to improve engraftment or to treat relapse, is problematic. The ability to detect the dominant unit might be useful in such cases. New strategies that are now being investigated include infusions of ex vivo expanded antiviral T cells and the generation of CIK cells or NK cells typically derived from small aliquots of residual cells obtained at time of cord blood infusion. Reliable and early prediction of the dominant unit would allow early selection of the appropriate starting cells to be further manipulated in the development of adoptive immunapies. Moreover, better donor selection through high-resolution HLA typing including HLA-C in addition to KIR gene content may improve outcomes and so move the field forward.

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